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Biodegradation of polystyrene by bacteria isolated from talabang tsinelas (*Crassostrea iredalei*) in Buguey Lagoon, Cagayan

Robelyn N. Marcos*[1], Jen Pearl L. Benarao[2], Danica Marie T. Pagatpatan[2], Mark Key John V. Sabio[2], Madel B. Tanguilan[2], Jefferson K. Soriano[2]

[1]*College of Natural Sciences and Mathematics, Cagayan State University, Carig, Tuguegarao City, Cagayan, Philippines*

[2]*Bureau of Fisheries and Aquatic Resources, Region 02, Carig, Tuguegarao City, Cagayan, Philippines*

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Abstract

The substantial surge in waste entering our aquatic ecosystems poses one of the leading environmental hazards. With the Philippines ranked as the third-largest contributor of plastic into the ocean annually, efforts to mitigate this issue through biodegradable alternatives are crucial. This study assessed the polystyrene (PS) biodegradation capability of microorganisms from talabang tsinelas (*Crassostrea iredalei*) in Buguey Lagoon, Cagayan. Two distinct colonies, *Pseudomonas aeruginosa* and *Enterobacter cloacae*, were identified using the API 20E biochemical test kit for their potential in polymer degradation. SEM analysis confirmed substantial surface deterioration of PS films exposed to these strains, indicating microbial degradation. One-way ANOVA analysis revealed no significant difference in polystyrene degradation among the isolated microorganisms, as all *p*-values exceeded the 0.05 significance level. The F-value for days 15, 30, and 45 were 1.207892, 1.324312, 1.665196, 1.665196, respectively, with corresponding *p*-values of 0.362385 for day zero (0), 0.333897 for day fifteen (15), 0.265922 on day thirty (30), and 0.167041 for day forty-five (45). These results indicate that the weight loss of polystyrene over time did not significantly differ between *P. aeruginosa and E. cloacae*. Results of the SEM also revealed the ability of *P. aeruginosa* and *E. cloacae*, to degrade PS plastic. The PS film subjected to *P. aeruginosa* exhibited the most extensive surface deterioration. This was characterized by the formation of deep pits, significant increases in surface roughness, and the development of cracks. Conversely, the PS film exposed to *E. cloacae* exhibited minimal or lesser degree of surface degradation compared to *P. aeruginosa*.

* **Corresponding Author:** Robelyn N. Marcos ✉ robelynmarcos02@gmail.com

Introduction

With the continuous advancement of science and technology alongside the expanding global population, the demand for plastics is on the rise. Ineffective management and disposal practices have led to substantial increase in plastic waste entering aquatic ecosystems, leading to significant environmental hazards. Each year, 4.8 to 12.7 million tons of plastic flow into our oceans, with Asia accounting for more than 80%. The Philippines is the third largest contributor, with an estimated 0.75 million metric tons of mismanaged plastic entering the ocean annually, about 20% of this plastic trash ends up in the ocean, damaging marine ecosystems and harming marine life (World Bank Group, 2021).

Plastics are man-made hazardous long chain synthetic polymers. One of the most inert synthetic polymers is Polystyrene (PS). In 2021, PS accounted for 5.3% of the 390 million tons of plastic produced. Expanded polystyrene (EPS) is most commonly used in aquaculture and takeout packaging boxes, and it causes significant pollution in coastal areas due to its low density, buoyancy, and waterproof qualities (Kaiser, 2010). EPS from source, including abandoned fishing gear and packaging materials, travels through rivers into the sea, accumulating in plastic waste patches PS is ingested by marine organisms, leading to bioaccumulation in multiple food chains (Pan *et al.*, 2022). Ingestion of plastic debris affects a wide range of marine species. Filter-feeding organisms, such as the Philippine cupped oyster, are vulnerable to ingesting contaminants from plastic either directly from plastic-polluted water or indirectly through contaminated planktonic prey because they filter enormous volumes of water every day to receive appropriate nourishment (Germanov *et al.*, 2018). Talabang tsinelas (*Crassostrea iredalei*) is the most commonly cultivated and harvested oyster species in the Philippines for human consumption. It is the most economically appealing since it grows larger quickly and has straight shell borders, making it simple to open (FAO, 2018).

Buguey was the top producer of oyster products in the province of Cagayan in 2016. The oyster culture has spread from Buguey to the surrounding towns of Santa Teresita and Gonzaga, as well as the eastern coastal towns of Sanchez Mira, Pamplona, and Claveria (Domingo, 2016). According to the Fisheries Statistics of the Philippines, the province's total oyster output in 2019 was 735.99 metric tons (MT), with a rise in production in 2021 to 787.26 MT. Plastic pollution, particularly from materials like PS, poses significant threats to marine ecosystems, wildlife, and human health. Two approaches to address plastic pollution are developing biodegradable plastics and employing microbes to degrade polymers into recyclable monomers (Wei *et al.* 2021). Analyzing polystyrene-degrading microbes in Talabang tsinelas (*C. iredalei*) in Buguey Lagoon, Cagayan, holds paramount importance due to the lagoon's significance in oyster production. Exploring the biodegradation capabilities of these bacteria, this study aimed to find natural solutions to mitigate plastic pollution. Furthermore, this endeavor enables us to potentially evaluate the associated risks to human health stemming from the consumption of such a vital local seafood resource.

Materials and methods

Materials

The following materials were used during the conduct of the study: analytical balance, hot plate, incubator, autoclave, drying oven, microscope, food blender, Petri dish, nutrient agar medium, mineral salt medium, and laboratory glassware.

Moreover, the following reagents were utilized: xylene solution, 70% ethanol. 3% saline solution, MSM (containing 2.27 g KH_2PO_4, 5.97 g Na_2HPO_4, 0.5 g NH_4Cl, 0.25 g $MgSO_4$, 0.0025 g $CaCl_2$, 0.001 $FeSO_4$, 0.0005 g $MnSO_4$, and 0.001 g

$ZnSO_4$ in 1 L distilled water), 2% aqueous sodium dodecyl sulfate (SDS) solution, and 0.05% mineral oil.

Research design

The study utilized Complete Randomized Design (CRD) to determine the differences between the treatments, while accounting for the differences among the day interval of incubation.

Treatments

T1 – Bacteria 1 + Mineral Salt Medium + polystyrene film

T2 – Bacteria 2 + Mineral Salt Medium + polystyrene film

Negative control – Sterile medium + polystyrene film

Management of test plates

Fig. 1. Lay-out for the PS biodegradation

Sampling method (Adopted from Jambre, 2021)

The study employed field sampling wherein samples of Talabang Tsinelas oyster (*C. Iredalei*) were collected from Buguey Lagoon, Cagayan. Following the procedure of Jambre (2021), the samples were then placed inside a sterile sampling bottle and were transferred to the laboratory immediately. Thereafter, the samples were processed in the laboratory of the Bureau of Fisheries and Aquatic Resources (BFAR).

Preparation of polystyrene (PS) powder (Adopted from Das and Kumar, 2014)

Following the procedure of Das and Kumar (2014), polystyrene foams were cut into small pieces and boiled with xylene for 15 minutes. After boiling, the pieces were crushed with a blender at 3,000 rpm for 10 minutes. The resulting polystyrene (PS) powder was washed with 70 % ethanol, dried overnight in a hot oven at 60ºC and stored at room temperature (Das and Kumar, 2014).

Enrichment and isolation of PS-degrading microorganisms (Adopted from Luis- Villaseñor et al., 2018 and Balasubramanian et al., 2010)

Following the procedure of Luis-Villaseñor *et al.* (2018), oysters were thoroughly washed and brushed under running tap water, and rinsed with sterile distilled water. The soft tissues were weighed (in grams) using analytical balance. The liquor and soft parts of oysters were homogenized for 90 seconds in 250 mL of 3 % saline solution using a sterile food blender.

Following the procedure of Balasubramanian *et al.* (2010), enrichment cultures were prepared by adding 10 mL suspension of homogenized oyster to 90 mL of Mineral Salt Medium (MSM). These enriched mediums were prepared in autoclaved flask by adding 2.27 g KH_2PO_4, 5.97 g Na_2HPO_4, 0.5 g NH_4Cl, 0.25 g $MgSO_4$, 0.0025 g $CaCl_2$, 0.001 $FeSO_4$, 0.0005 g $MnSO_4$, and 0.001 g $ZnSO_4$ in 1 L distilled water. The medium was used in this study so that plastic was the only carbon source for bacteria (Bakht *et al.*, 2020). The solution was supplemented with 300 mg of PS powder. The samples were then incubated for 5 days at 28°C.

After incubation, samples taken from the enrichment culture were serially diluted and were spread plated in nutrient agar and subsequently purified using streak method. All plates were incubated at 37°C for 24 hours. All pure isolates were maintained in a nutrient agar broth.

API 20E method

Samples of bacterial isolates were subjected for identification using the API 20E biochemical test and were further processed at the laboratory of Bureau of Fisheries and Aquatic Resources RO2.

Screening of polystyrene (PS)-degrading microorganism (Adopted from Pramila- Ramesh, 2011 and Das-Kumar, 2014)

Bacterial isolates were tested for PS-degrading efficiency following the procedure of Pramila and Ramesh (2011). Flasks containing 50 ml of MSM were amended with pre-weighed PS film. Prior to aseptic transfer to liquid media for the biodegradation assay, the polystyrene films (1.5 cm by 1.5 cm) were disinfected for 30 minutes in 70% ethanol, air-dried for 15 minutes in the laminar airflow chamber, and treated with mineral oil (0.05%) to allow bacterial adhesion (Das and Kumar, 2014). The flasks were added with 1 ml of a 24-hour bacterial culture maintained at a turbidity equivalent to 0.5 McFarland standards. The same set-up without bacterial isolate was used as the negative control. All set-ups in duplicate were incubated at 37°C for 45 days. The weight of the residual PS was determined every 15 days of incubation.

Determination of dry weight of residual PS (Adopted from Gilan et al., 2004)

In order to facilitate accurate measurement of the dry weight of residual PS, recovered PS films were washed with 2% (v/v) aqueous sodium dodecyl sulfate (SDS) solution for 4 hours and then with distilled water (Gilan *et al.*, 2004). The mineral oil added on the PS films was removed using ethanol and then SDS wash. The washed PS films were dried overnight on a filter paper at 60°C before weighing. The percentage of weight loss was calculated using the following equation:

Percentage of weight loss = [(Initial weight - Final weight) / Initial weight] × 100

Surface visualization using scanning electron microscope

PS films from the control and treatment groups were randomly selected and submitted at ADMATEL (Advance Device and Materials Testing Laboratory), DOST Cpd, General Santos Avenue, Bicutan, Taguig City for SEM Viewing. Submitted samples were viewed using Scanning Electron microscope to visualize the surface of each film after exposing it to the PS-degrading microorganisms.

Flow chart

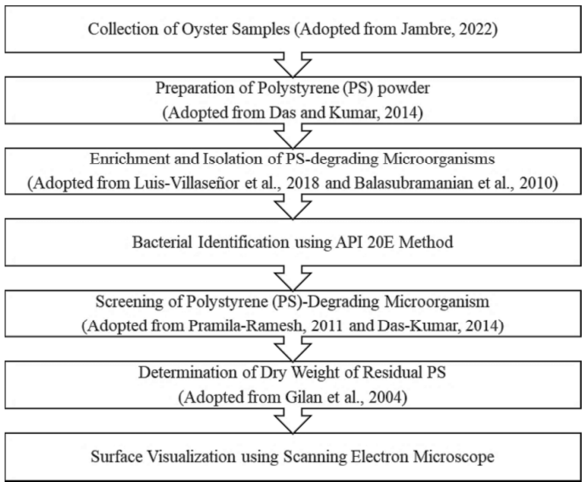
Collection of Oyster Samples (Adopted from Jambre, 2022)
Preparation of Polystyrene (PS) powder
(Adopted from Das and Kumar, 2014)
Enrichment and Isolation of PS-degrading Microorganisms
(Adopted from Luis-Villaseñor et al., 2018 and Balasubramanian et al., 2010)
Bacterial Identification using API 20E Method
Screening of Polystyrene (PS)-Degrading Microorganism
(Adopted from Pramila-Ramesh, 2011 and Das-Kumar, 2014)
Determination of Dry Weight of Residual PS
(Adopted from Gilan et al., 2004)
Surface Visualization using Scanning Electron Microscope

Fig. 2. Schematic diagram of the methodology

Statistical analysis

Experimental results were expressed as percentage on the weight loss of polystyrene films. Tests of significance of the differences between treatments were determined using one-way ANOVA. At a 95% confidence level (significance level of $\alpha=0.05$), p-value < 0.05 was used to indicate whether there are significant differences between the treatments.

Results and discussion

Isolation and identification of PS-degrading microorganisms

Table 1 shows the Identification of isolated microorganisms. Among the isolates, one was identified as *Pseudomonas aeruginosa* with a code of "B1", a Gram-negative, rod-shaped bacterium that exhibits notable metabolic versatility. *P. aeruginosa* is renowned for its ability to thrive in various environments, including soil, water, and human tissues. It can metabolize a wide range of organic compounds, a trait that supports its potential role in polystyrene (PS) degradation. The metabolic flexibility of *P.*

aeruginosa is particularly advantageous for biodegradation processes, as it can utilize various carbon sources derived from PS breakdown (Pathak *et al.*, 2017). Furthermore, *P. aeruginosa* is known for its robust biofilm-forming abilities on PS surfaces. Biofilm formation is critical because it concentrates enzymatic activity in localized microenvironments, enhancing the efficiency of degradation processes (Moradali *et al.*, 2017). Within these biofilms, interactions between *P. aeruginosa* and the PS substrate are optimized, facilitating the enzymatic degradation and subsequent metabolic utilization of PS-derived carbon sources.

Table 1. Bacterial identification of PS- degrading microorganisms isolated from *Crassostrea iredalei*

Sample	Method of identification	Microorganisms
B1	API20E/Biochemical testing	*Pseudomonas aeruginosa*
B2	API20E/Biochemical testing	*Enterobacter cloacae*

Additionally, the other isolate was identified as *Enterobacter cloacae* with a code of "B2", another gram-negative, rod-shaped bacterium known for its significant metabolic adaptability. *E. cloacae* can thrive in diverse environmental conditions and degrade a variety of organic compounds. It is commonly found in soil, water, sewage, and the intestines of animals. Its role in PS degradation adds a layer of complexity to the microbial consortia involved in these processes. *E. cloacae* is well-documented for its ability to produce a variety of enzymes, including hydrolases and oxidoreductases, which can break down complex polymers (Mohanan *et al.*, 2020). This bacterium is known for its ability to degrade various environmental pollutants, including hydrocarbons and pesticides, which underscores its potential in bioremediation (Jiang *et al.*, 2012).

E. cloacae is also capable of forming biofilms, similar to *P. aeruginosa*, which aid in the concentration of degradation activity and the protection of bacterial cells from environmental stressors. These biofilms facilitate the cooperative breakdown of complex substrates such as PS. Research has shown that E. cloacae can degrade other synthetic polymers, suggesting a broad enzymatic capability that can be harnessed for PS degradation (Prakash *et al.*, 2014).

In -vitro biodegradation assay

Fig. 3 showed the percentage weight loss of PS films in every 15 days of incubation with the bacterial isolates compared to the untreated samples.

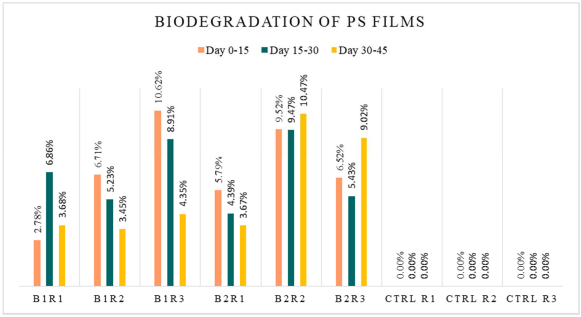

Fig. 3. Percentage weight loss of PS films in every 15 days of incubation with the bacterial isolates compared to the untreated samples

The results showed the bacterial isolates from Talabang Tsinelas (*Crassostrea iredalei*) related to *Enterobacter cloacae* and *Pseudomonas aeruginosa* were capable of utilizing polystyrene (PS) as the sole carbon source. The two isolates were capable of polystyrene degradation based on weight loss of the films in every 15 days of incubation for 45 days. Both B1 and B2 isolates showed a significant difference relative to the negative control.

After the first 15 days of incubation, B1 of the third replicate has shown the highest calculated percentage weight loss. The percentage weight loss of PS films was calculated as 10.62%. Meanwhile, B2 of the second replicate has shown the highest calculated percentage weight loss. The percentage weight loss of PS films was calculated as 9.52%. On the second 15 days of incubation, B1 of the third replicate has shown the highest

calculated percentage weight loss. The percentage weight loss of PS films decreased by 1.71%, from 10.62% to 8.91%. Meanwhile, B2 of the second replicate has shown the highest calculated percentage weight loss. The percentage weight loss of PS films was decreased by 0.05%, from 9.52% to 9.47%. The percent decrease in the weight loss of PS films showed a slow degradation rate of the two isolates. On the third 15 days of incubation, B1 of the third replicate has shown the highest calculated percentage weight loss. The percentage weight loss of PS films rapidly decreased by 4.56%, from 8.91% to 4.35%. Meanwhile, B2 of the second replicate has shown the highest calculated percentage weight loss. The percentage weight loss of PS films was increased by 1%, from 9.47% to 10.47%. The percent decrease in the weight loss of PS films showed a very slow degradation rate of *E. cloacae*, whereas *P. aeruginosa* showed a faster degradation rate based on the percent increase in the weight loss of the film.

Table 2. Total biodegradation of PS films based on the percentage weight loss after 45 days of incubation with the bacterial isolates compared to the untreated samples

Treatments	Initial weight	Final weight	% Weight loss
B1R1	0.018	0.0157	12.7778%
B1R2	0.0164	0.014	14.6341%
B1R3	0.0113	0.0088	22.1239%
B2R1	0.0121	0.0105	13.2231%
B2R2	0.0105	0.0077	26.6667%
B2R3	0.0138	0.0111	19.5652%
CTRL R1	0.0145	0.0145	0.0000%
CTRL R2	0.0123	0.0123	0.0000%
CTRL R3	0.0164	0.0164	0.0000%

Table 2 shows the total biodegradation of PS films based on the percentage weight loss after 45 days of incubation with the bacterial isolates compared to the untreated samples. Between the two isolates that were identified, under B1 of the third replicate *P. aeruginosa* showed a lower total biodegradation with a calculated percentage weight loss of 22.12% than B2 of the third replicate *E. cloacae* which showed a calculated percentage weight loss of 26.67%.

In general, these microorganisms utilized hydrocarbons present in the polymer backbone, making them the key players of biodegradation. Mixed populations of bacterial communities are usually involved in the biodegradation process, which includes the Pseudomonas and *Enterobacter* sp. These bacteria have the ability to degrade hydrocarbons and various organic molecules in polystyrene with the use of enzymes like monooxygenase, dioxygenase, and dehydrogenase (Kyaw *et al.*, 2012; Bhatia *et al.*, 2014).

Table 3. Descriptive table indicating the mean weight loss of PS films in every 15 days of incubation with the bacterial isolates compared to the untreated samples

Treatments	DAY 0 - Means	DAY 15 - Means	DAY 30 - Means	DAY 45 - Means
T1	0.015233	0.0143	0.013333	0.012833
T2	0.012133	0.011267	0.010567	0.009767
CTRL	0.0144	0.0144	0.0144	0.0144
All Grps	0.013922	0.013322	0.012767	0.012333

Table 3 shows the mean weight loss of PS films in every 15 days of incubation with the bacterial isolates compared to the untreated samples. The table above indicates the mean weight loss of Treatment 1, Treatment 2, and the Control Group, with Treatment 1 having a computed mean value of 0.015233 for day 0, 0.0143 for day 15, 0.013333 for day 30, and a mean of 0.012833 for day 45. Treatment 2 revealed a computed treatment mean of 0.012133 for day 0, 0.011267 for day 15, 0.010567 for day 30, and a treatment mean of 0.009767 for day 45. The Control group revealed a treatment mean of 0.0144 on day 0 until day 15.

Table 4 shows the ANOVA of the mean weight loss of polystyrene plastic after 15, 30, and 45 days. The table above indicates the degradation of polystyrene plastic, facilitated by isolated microorganisms from Talabang tsinelas (*Crassostrea iredalei*) starting from day 0 until day 45. Based on the table, the f-value computed for day 0 revealed a value of 1.207892 and a p-value of 0.362385. An f-

value of 1.324312 and a p-value of 0.333897 was documented for day 15. Over the subsequent fifteen days (30th day), an f- value of 1.665196 and a p-value of 0.265922 was revealed, and an f-value of 2.447291 and a p-value of 0.167041 was recorded for day forty-five. Upon computation, since the tabulated p-values of Treatment 1, Treatment 2, and the Control group starting from day 0 until day 45 were greater than the set significance level of .05000, it was revealed that there was no significant difference among the treatment groups and the weight loss measurements of the polystyrene plastic over the course of forty-five (45) consecutive days, each interval comprising fifteen (15) days.

Table 4. Determination of the mean weight loss of Polystyrene Plastic after 15, 30, and 45 days

	SS-effect	df- Effect	MS-Effect	SS-Error	df- error	MS-Error	F	p	sig.
DAY 0	0.000015	2	0.000008	0.000038	6	0.000006	1.207892	0.362385	ns
DAY 15	0.000019	2	0.00001	0.000043	6	0.000007	1.324312	0.333897	ns
DAY 30	0.000023	2	0.000012	0.000042	6	0.000007	1.665196	0.265922	ns
DAY 45	0.000033	2	0.000017	0.000041	6	0.000007	2.447291	0.167041	ns

*marked effects are significant at p < .05000

Scanning electron microscopy (SEM) analysis

SEM analysis was employed to investigate the degradation of PS film surfaces by various bacterial strains. After a 45-day incubation period with each isolate, PS film samples were randomly selected for examination under SEM.

The SEM analysis revealed the ability of several strains, particularly *Pseudomonas aeruginosa* and *Enterobacter cloacae*, to degrade PS plastic. The Polystyrene (PS) film subjected to *P. aeruginosa* exhibited the most extensive surface deterioration. This was characterized by the formation of holes and the development of cracks (Yang *et al.*, 2020a, 2015b). Conversely, the PS film exposed to *E. cloacae* exhibited minimal or lesser degree of surface degradation compared to *P. aeruginosa*.

These observable changes strongly suggest microbial degradation of PS, which is supported by previous research (Yang *et al.*, 2020a, 2015b). Additionally, the observed structural transformations and surface colonization by microbial communities serve as initial indicators of plastic biodegradation, aligning with earlier findings (Joshi *et al.*, 2022). Furthermore, prior studies have documented the capability of *E. cloacae* for polymer degradation (Sarker *et al.*, 2020). According to Asmita *et al.* (2015) study, *P. aeruginosa* is another bacterium exhibiting potential for polystyrene plastic degradation.

The PS films exposed to bacterial strains displayed significant surface deformations compared to the control film. These observations suggest that the *P. aeruginosa* and *E. cloacae* degraded and fragmented the PS film. In contrast, the control PS film, which lacked bacterial exposure, maintained an unaltered surface morphology.

Fig. 4 shows the observations of the deterioration of PS film surface topography after 45-day incubation through Scanning Electron Microscopy (SEM) Imaging.

The SEM images indicated evident deterioration of the PS surface caused by *P. aeruginosa*. The degradation of *P. aeruginosa* manifested as the formation of holes indicated by a red circle and cracks indicated by a yellow circle. Similar to *P. aeruginosa*, *E. Cloacae* also demonstrated the ability to degrade the PS plastic surface of the film. SEM analysis in *E. cloacae* identified the presence of the initiation of degradation indicated by a yellow arrow and pits indicated by a blue circle on the PS film incubated with *E. cloacae*.

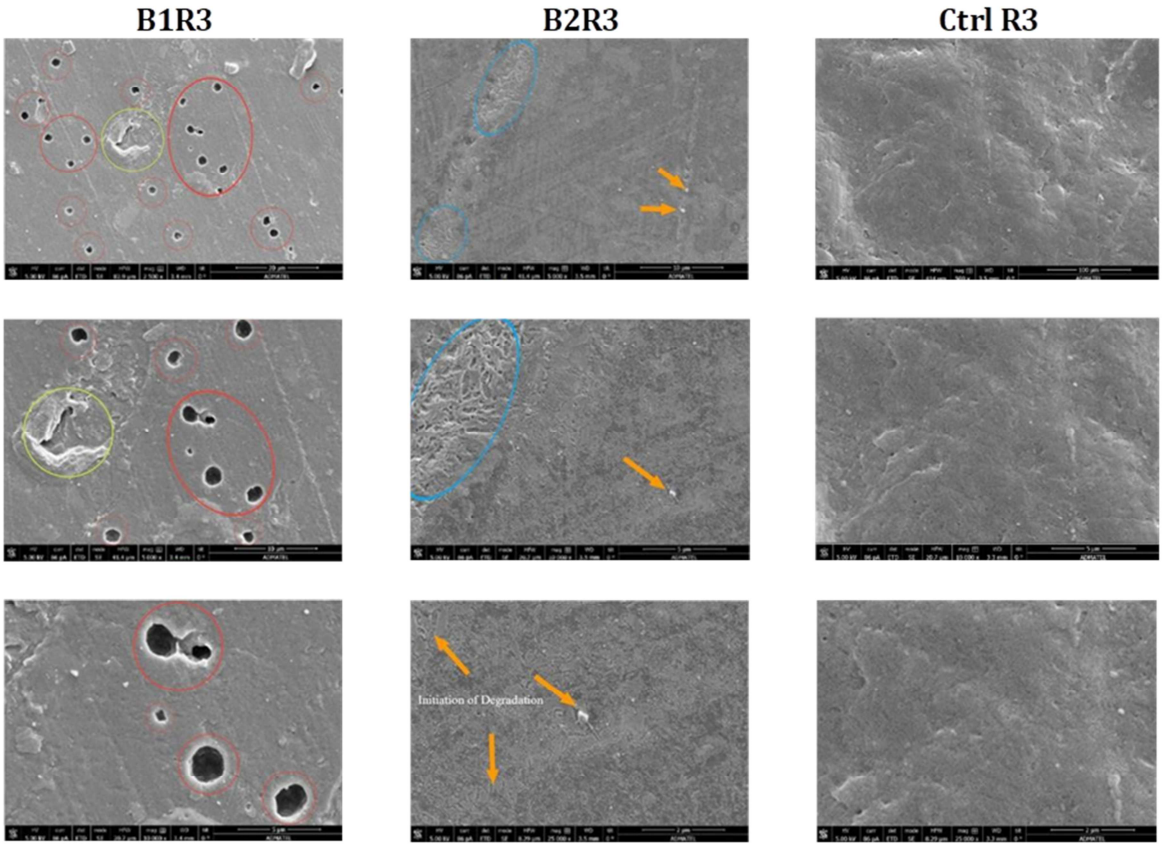

Fig. 4. SEM observations of the deterioration of PS film surface topography after a 45-day incubation with the strains *P. aeruginosa* (B1R3) and *E. cloacae* (B2R3). The physical surface topography of the uninoculated control showed no observed surface deterioration

The uninoculated control sample exhibited no observable surface deterioration by SEM analysis, confirming the absence of degradation in the absence of bacterial exposure.

The SEM analysis revealed clear morphological changes, indicative of the degradation in the plastic film by two bacteria. However, the weight loss measurements do not show statistically significant different between the treatments. The findings suggest that over the duration of the experiment, the efficacy of the isolated microorganisms from *C. iredalei* in degrading polystyrene plastic did not significantly vary.

Conclusion

Based on the findings of the study, the following conclusion is drawn:

Polystyrene (PS), a widely used plastic in human activities, poses significant environmental challenges due to its resistance to degradation. This study is a pioneering inquiry into the biodegradation of PS using indigenous aquatic bacteria obtained from *Crassostrea iredalei* in Buguey Lagoon, Cagayan. The isolation and identification of *Pseudomonas aeruginosa* and *Enterobacter cloacae* from *C. iredalei* underscores the complexity and efficiency of microbial communities involved in PS degradation to mitigate PS pollution in aquatic environments.

The biofilm-forming ability of *P. aeruginosa* and the metabolic versatility of *E. cloacae* collectively contribute to the breakdown and utilization of PS, offering promising insights for bioremediation strategies aimed at mitigating plastic pollution. This approach harnesses the natural resilience of microbial communities and emphasizes the importance of preserving local biodiversity for environmental conservation.

Recommendations

In light with the findings and conclusions derived from this study, the following recommendations are hereby presented:

1. Utilize molecular techniques like 16S rRNA gene sequencing to identify and classify isolated microorganisms accurately, potentially discovering new strains capable of degrading PS, alongside employing Gel Permeation Chromatography to observe changes in the polymer's molecular weight distribution, providing insights into degradation progress.
2. Quantify plastic degradation by measuring changes in plastic weight relative to the colony count of isolated species, establishing a correlation between microbial growth and plastic breakdown.
3. Investigate the impact of biofilm formation on degradation efficiency and explore methods to enhance biofilm development on plastic surfaces, employing atomic force microscopy (AFM) alongside scanning electron microscopy (SEM) to observe physical changes and degradation extent at the microscopic level.
4. Explore different environmental conditions (e.g., temperature, pH, nutrient availability) to optimize the degradation process. Conduct experiments to determine the optimal conditions for maximum microbial activity and PS degradation.
5. Perform a power analysis to determine whether increasing replicates could yield statistically significant results, while also employing respirometry to measure CO_2 production as an indirect indicator of microbial activity and plastic breakdown.
6. Conduct long-term degradation studies to assess the persistence and efficacy of degradation over extended periods, providing insights into real-world application sustainability, and utilizing gas chromatography-mass spectrometry (GC-MS) or Fourier-transform infrared spectroscopy (FTIR) to identify degradation products and assess chemical composition alterations.
7. Investigate the synergistic effects of mixed microbial cultures on PS degradation, as co-culturing different bacterial species may enhance degradation efficiency through complementary metabolic pathways, while also evaluating the environmental impact of the degradation process by analyzing by-products to ensure they are non-toxic and environmentally safe.

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and glorify Him for the wisdom, knowledge, and strength He has bestowed upon them.

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